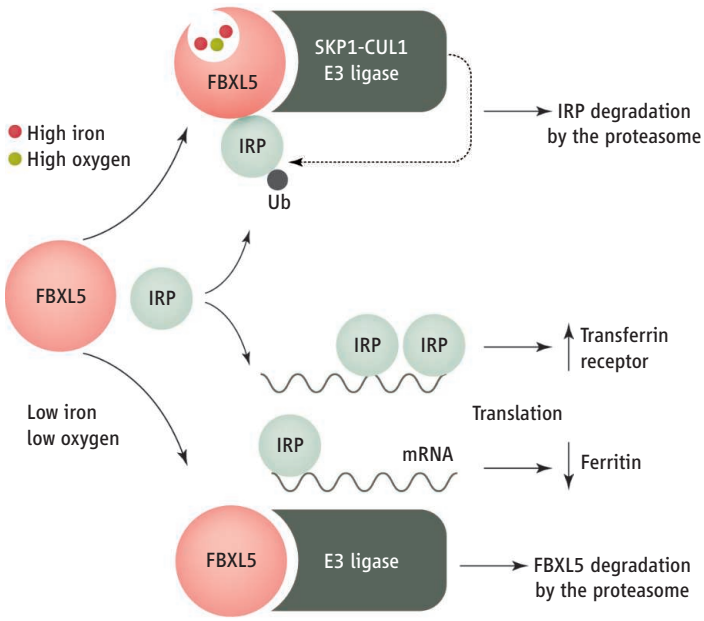
CELL BIOLOGY

An Ancient Gauge for Iron

A protein with a domain that binds to oxygen and iron acts as a sensor to control iron metabolism in human cells.

Tracey A. Rouault

Mammalian cells must manage the import, export, and sequestration of iron to achieve the cytosolic concentrations needed to support the synthesis of iron-binding proteins and prevent unfavorable iron-dependent oxidation events. Key to this maintenance are the iron regulatory proteins IRP1 and IRP2, which respond to the cytosolic iron pool by binding to target mRNA and regulating the synthesis of iron metabolism proteins (*1*–*3*). On pages 718 and 722 of this issue, Vashisht *et al*. and Salahudeen *et al*. (*4*, *5*) report that human cells gauge cellular iron and concomitantly alter the activity of IRPs through a mechanism that depends on the protein FBXL5. FBXL5 senses iron through an evolutionarily conserved hemerythrin domain that is related to a family of iron- and oxygen-binding proteins in bacteria and invertebrates.



Regulation of iron homeostasis. The concentrations of iron and oxygen in mammalian cells determine the stability of FBXL5, which consequently determines whether IRPs are degraded by the ubiquitin (Ub)-proteasome system or kept available to control the expression of target mRNAs. These mRNAs encode proteins important in cellular iron homeostasis, including ferritin (an iron sequestration protein) and the transferrin receptor (an iron uptake protein). FBXL5 likely contains an oxygen-bridged di-iron binding site within a conserved hemerythrin domain. IRPs bear motifs that are targets for FBXL5, but the accessibility of these motifs may determine the efficiency of FBXL5-mediated degradation.

The role of FBXL5 in iron sensing was discovered through two approaches. Vashisht *et al*. focused on identifying new roles for mammalian F-box proteins. The F-box is a 42– to 48–amino acid motif composed of three α helices that form a pyramidal shape. The human genome includes more than 20 proteins that contain both an F-box (*6*) and a domain of leucine-rich repeats (FBXL) that provides the architecture for protein-protein interactions (*7*). An F-box protein tethers a target protein to an E3 ligase complex that tags the target with ubiquitin molecules, thereby marking it for degradation by the proteasome (*8*). To identify new targets of FBXL5, its F-box was deleted and the resulting protein (which could avoid degradation) was expressed in cultured human cells. IRP1 and IRP2 were identified (by mass spectrometry) as FBXL5 binding proteins.

In a different approach, Salahudeen *et al*. used RNA interference to decrease the expression of E3 ligase components in cultured human cells. When cells were treated with iron, IRP2 degradation was observed. However, IRP2 was spared from degradation in cells that lost expression of FBXL5 or any of the components of the SCF class of multimeric E3 ligases (which contain the proteins Skp1, Cullin 1, and RBX1) (*8*).

Unexpectedly, both groups identified a conserved hemerythrin domain at the N terminus of FBXL5. Previously, hemerythrins were recognized as oxygen-carrying proteins in invertebrates and as potential oxygen sensors in bacteria, but were not known to exist in higher life forms (*9*). Hemerythrin consists of a four–α helix barrel structure in which an active site is formed by two iron atoms ligated to residues from all four helices, and bridged by one oxygen atom from the solvent (see the figure). Molecular oxygen binds to one of the iron atoms; upon binding, each iron atom donates an electron to the oxygen molecule to form a hydroperoxide, which is stabilized by the surrounding protein sheath (*9*, *10*). Thus, dioxygen binding and the concomitant oxidation of the two bound iron atoms increases the iron-binding affinity and stability of the hemerythrin domain.

Both groups report that the binding of iron and oxygen to the hemerythrin domain stabilizes FBXL5, whereas a lack of iron (or lack of oxygen in the presence of sufficient iron) results in degradation. Deletional analyses of FBXL5 established that the N-terminal 161 amino acids were required for iron-dependent degradation (*5*). In addition, the C-terminal region of FBXL5, which contains the leucine-rich repeats, binds to IRPs (*4*, *5*). Because iron and oxygen stabilize FBXL5, targeting of IRPs for degradation occurs in cells that are iron-replete. Thermal denaturation experiments of the N-terminal 161–amino acid fragment suggest that removal of iron leads to unfolding of the hemerythrin domain (*5*), which likely exposes FBXL5 to attack by yet another specific E3 ligase (*4*, *5*). These discoveries reveal that FBXL5 directly interacts with iron, enabling it to sample iron levels in real time, and that iron stabilizes FBXL5, allowing it to target IRPs for degradation.

Although both IRP1 and IRP2 are targets for FBXL5, there is another layer of regulation for IRP1 that protects it from iron-dependent degradation. In cells that are rich in iron, IRP1 ligates an iron-sulfur cluster and functions as an aconitase, interconverting citrate and isocitrate (*1*). The presence of the iron-sulfur cluster likely drives a conformational change in IRP1 (*11*) that limits accessibility of the "degron," the sequence(s) on target proteins to which FBXL5 binds. When cells are low in iron, IRP1 loses its iron-sulfur cluster and undergoes a conformational change that enables it to bind to sequences in mRNA known as iron-responsive elements (IREs) (*1*–*3*). Similarly, in iron-depleted cells, IRP2 also binds to IREs.

The IRE-binding activity of IRP1 and IRP2 differentially controls the transla-

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tion of mRNAs. For example, when iron is low, IRPs inhibits the translation of mRNA encoding the cytosolic iron-binding protein ferritin. This reduces iron sequestration, making it available for cellular processes. IRP binding also protects mRNA encoding the transferrin receptor (which transports iron into cells) from degradation, and consequently boosts its expression when the concentration of cytosolic iron is low. As expected, manipulations of FBXL5 activity affected the amounts of ferritin (*4*) and transferrin receptor mRNA in cells (*5*). Thus, the activity of the IRE-IRP regulatory system is controlled by FBXL5, which directly reflects cellular iron status through the iron binding of its conserved hemerythrin domain (*10*).

In the parsimonious evolutionary process, hemerythrin was surpassed by heme as the oxygen carrier of choice (*12*). However, it seems that in higher life forms, the hemerythrin domain was successfully repurposed as an iron sensor to function in cellular iron homeostasis.

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BIOCHEMISTRY

Leaps in Translational Elongation

Electron microscopic and crystallographic studies shed light on key steps in the protein synthesis process in ribosomes.

Anders Liljas

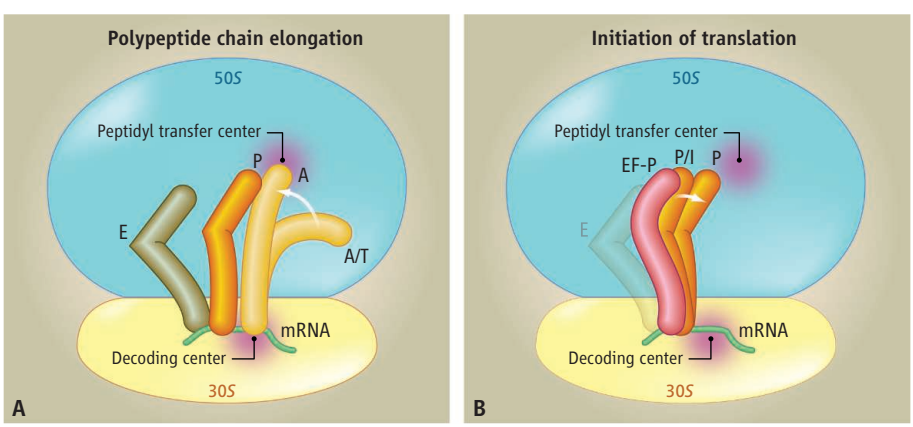
Ribosomes are the sites of protein synthesis in all cells. They are complexes of one or two small and two large RNA molecules and a multitude of proteins and are divided into a small and a large subunit. This complex system, with its many functional steps, cannot be described in a cartoon fashion. Each year, further details of the complexity of protein synthesis emerge. Two Research Articles in this week's issue (*1*, *2*) and two other recent studies (*3*, *4*) shed light on the mechanisms, dynamics, and functions of key elements in the protein synthesis process.

To synthesize new proteins, the ribosome translates the information encoded in messnger RNA (mRNA) into a chain of amino acids. New amino acids are delivered by a transfer RNA (tRNA) called aminoacyl-tRNA to the A site (see the figure, panel A). In the decoding center, the ribosome ensures that the codon (a sequence of three nucleotides that specifies an amino acid) of the mRNA matches the anticodon of the tRNA and that the correct amino acid is thus inserted. Next, the peptidyl-tRNA in the P site donates its growing polypeptide to the amino acid on the tRNA in the A site. The newly formed peptidyl-tRNA is then translocated from the A to the P site. Simultaneously, the empty tRNA in the P site is moved into the exit or E site.

This protein synthesis process would be very inefficient without the catalytic participation of translation factors and without the small molecule guanosine 5′-triphosphate (GTP). Some translation factors are enzymes of the family guanine triphosphatases (GTPases), which bind and hydrolyze GTP to guanosine diphosphate (GDP). The elongation factor Tu (EF-Tu), in complex with GTP and an aminoacyl-tRNA, delivers the tRNA into the A site of the ribosome. Translocation of the newly formed peptidyl-tRNA is catalyzed by elongation factor G (EF-G) in complex with GTP.

During the polypeptide chain elongation cycle, the ribosomal subunits rotate with respect to each other to assist the movement of the tRNAs. This "ratcheting" process (*5*) has so far been studied mainly by single-particle reconstruction with low-temperature electron microscopy (cryo-EM). Cate and co-workers (*3*) recently described the crystallographic details of ratcheting, including intermediate stages. They identified four ribosomal conformations, corresponding to different stages in the translation process. In addition to the rotation of the subunits, the head domain of the small subunit swivels in a coordinated fashion. The contacts between the subunits undergo strain or change during ratcheting.

It has not been possible to study the ribosomal binding of translational GTPases by crystallography, because their binding site was occupied by the L9 protein of a neighboring ribosome in the crystals. Good structural information about ribosome-bound GTPases is available from cryo-EM (*6*–*8*), but the higher



Essential steps in translation. (**A**) The two ribosomal subunits 30S (yellow) and 50S (blue), with the decoding center and the peptidyl transfer center. The mRNA (green) binds to the 30S subunit. Four different positions for tRNA molecules are indicated: The A site binds aminoacyl-tRNA, the P site binds peptidyl-tRNA, and the E site binds exiting tRNA. Schmeing *et al*. (*1*) show that the bent tRNA in the A/T site is bound together with EF-Tu. A movie showing an animation of decoding by the ribosome and EF-Tu is available in Schmeing *et al*. (movie S1) at www.sciencemag.org/cgi/content/full/1179700/DC1. (**B**) The initiator tRNA first binds to the P/I site. Binding of EF-P on the E-site side of the tRNA moves the tRNA to the P site (*4*).

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